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Antioxidant activity of the mangiferin inclusion complex with β-cyclodextrin

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ABSTRACT

The aim of this study was to characterize the complex between mangiferin (MGN) with β-cyclodextrin (β-CD) obtained through the co-evaporation method using Differential Scanning Calorimetry (DSC) and Fourier Transform Infrared spectroscopy (FT-IR), as well as to verify the antioxidant activity of this complex by means of radical scavenging activity toward 2,2′-diphenyl-1-picrylhydrazyl radical (RSA-DPPH•), with additional analysis of solvent effects, and Oxygen Radical Antioxidant Capacity-Fluorescein (ORAC-FL) assays. The protective effects of MGN and the MGN:β-CD complex against peroxyl radical-initiated membrane lipid peroxidation were also evaluated. The analysis of the solvent effects in the DPPH• method demonstrated that at amounts of organic solvents lower than the ratio 50:50 (methanol–water or ethanol–water) there is a drawback, due to the hydrophobic nature of DPPH• and should not be used. The antioxidant activity of the MGN:β-CD complex was higher than in its free form, as seen by both methods. The RSA-DPPH• value was twofold larger for the complex while the ORAC-FL value was fifteenfold larger. Lipid peroxidation assays showed that the MGN:β-CD complex has a protective effect on the membrane that is as effective as the positive control (Trolox).

1. Introduction

Mangiferin (1,3,6,7-tetrahydroxy-2-[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]xanthen-9-one) (MGN) (Fig. 1) is a naturally occurring polyphenol in several fruits, one being *Mangifera indica* L. (common name: mango), one of the most popular tropical fruit-bearing trees in the world (Barreto et al., 2008). The interest in MGN stems from its wide range of biological actions, for instance, gastroprotective (Carvalho et al., 2007), analgesic (Dar et al., 2005), antibacterial (Duang, Wang, Zhou, & Huang, 2011) together with cytoprotective (Pardo-Andreu et al., 2006). The therapeutic potential of MGN has been investigated in the prevention and treatment of periodontitis (Carvalho et al., 2009). A wide spectra of these properties have been attributed to its antioxidant properties, being MGN the major component (10–20%) of the aqueous formulation named Vimang® used in Cuba (Garrido, González, Romay, Núñez-Sellés, & Delgado, 2008). However this antioxidant activity does not necessarily lead to an in vivo biological effect implying poor bioavailability, due to its low solubility in water: 0.111 mg/mL (Van der Merwe et al., 2012; Wang, Deng, Li, & Wang, 2007). This problem can be minimized by using cyclodextrins (CDs), that present special ability to complex with a variety of guest molecules, which enables their solubility, stability, bioavailability, protection against light-induced decomposition, to suppress unpleasant odors or tastes and achieve a controlled release of certain constituents (Astray, Gonzalez-Barreiro, Mejuto, Rial-Otero, & Simal-Gándara, 2009), and still increase the antioxidant activity of many compounds (Lu, Cheng, Hub, Zhang, & Zou, 2009).

Several studies have been conducted in search of natural antioxidants for food preservation in place of BHT (butylated hydroxytoluene), that may be responsible for liver damage and carcinogenesis (Krishnaiah, Sarbatly, & Nithyanandam, 2011). An alternative to this problem is the supplementation of foods and liquid drinks with natural antioxidants complexed with cyclodextrin (Basu & Del Vecchio, 2001). Thus, the MGN:β-CD complex may have future application in the food, pharmaceutical and cosmetic industries.

The complexation of MGN in β-cyclodextrin (β-CD) has been described by our group, its stoichiometry determined as 1:1 and its apparent formation constant (K_F) was calculated using the Benesi–Hildebrand method and by cyclic voltammetry (Ferreira et al., 2010). Other studies (Huang, He, Lu, Ge, & Guo, 2011; Teng, Yu, Zhai, Li, & Liu,

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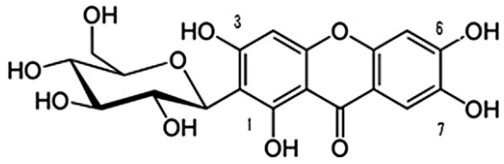


Fig. 1. Structure of mangiferin (MGN).

2007; Zhang et al., 2010) also show that the inclusion of MGN on the CDs cavity increases its solubility and bioavailability. However, it is still unknown whether entrapment in the internal cavity of CDs affects the antioxidant activity of MGN. Thus, the aim of this study was to characterize the MGN:β-CD complex and to evaluate its antioxidant activity, using radical scavenging activity toward 2,2′-diphenyl-1-picrylhydrazyl radical (RSA-DPPH$^{\bullet}$) and Oxygen Radical Antioxidant Capacity (ORAC) assay using Fluorescein as a probe molecule. In addition, its protective effect against peroxyl radical-initiated membrane lipid peroxidation was evaluated.

2. Materials and methods

2.1. Materials

DPPH$^{\bullet}$ (2,2′-diphenyl-1-picrylhydrazyl radical), two types of β-cyclodextrin (β-CD) [CAS Number 7585-39-9 (for DSC studies) and CAS Number 68168-23-0], Fluorescein disodium salt (FL), Trolox, soy phosphatidylcholine and AAPH were purchased from Sigma–Aldrich (St. Louis, USA). Gallic acid (GA) was obtained from Vetec Química Fina Ltda. (Rio de Janeiro, Brazil) and the fluorescent fatty acid-analog, 4,4-difluoro-5-(4-phenyl-1,3-butadienyl)-4-bora-3a,4a-diaza-S-indacene-3-undecanoic acid (C_{11}-BODIPY$^{581/591}$), from Molecular Probes (Ontario, Canada). MGN (Fig. 1) was obtained from an ethanolic extract prepared from dried bark of *M. indica*, recrystallized in aqueous ethyl acetate and characterized (Barreto et al., 2008). All the reagents were of analytical grade.

2.2. Preparation of the complexes

The physical mixture of MGN+β-CD, in an equimolar ratio (1:1), was prepared in an agate mortar, mixing them until homogenization. The complex (1:1) was obtained by co-evaporation. MGN and β-CD, in an equimolar ratio (1:1) were added to an aqueous solution prepared with 5 mL ethanol/100 mL water. The solution was protected from light and mechanically shaken at 170 rpm at 25 °C in a Marconi MA-420 incubator shaker (São Paulo, Brazil) for 24 h. After evaporation of the ethanol from the reaction mixture, the uncomplexed MGN was removed by filtration. Then, water was evaporated under reduced pressure in a Büchi Rotavapor (Büchi, Germany) and dried in vacuum, giving the MGN:β-CD complex.

2.3. Physicochemical characterization of MGN:β-CD complex

2.3.1. Fourier Transform Infrared spectroscopy (FT-IR) and Differential Scanning Calorimetry (DSC)

The FT-IR spectra of MGN, β-CD and MGN:β-CD complex were recorded at room temperature in a spectral region between 4000 and 500 cm^{-1} on an IRPrestige-21, Fourier Transform Spectrometer (Shimadzu, Kyoto, Japan). Samples were prepared as small pellets by mixing each of them in a mortar with KBr (1:100) and then pressed. A blank KBr disc was used as a background. DSC analysis was carried out for MGN, β-CD and the complex with a DSC-60 calorimeter (range 25–500 °C) (Shimadzu, Kyoto, Japan). The temperature scale was calibrated using α-alumina powder. Samples (5.0–10.0 mg) were placed in standard aluminum pans and measurements were performed at a heating rate of 5 °C min^{-1} from 25 to 400 °C in a dynamic nitrogen atmosphere (flow rate = 20 mL/min).

2.4. Sample preparations for analysis of antioxidant activity

The MGN and MGN:β-CD complex were prepared with 5 mL ethanol/100 mL water. The solution of the MGN:β-CD (1:1) complex was prepared at concentrations of 50, 100 and 500 μmol L^{-1}. The solutions were stirred (170 rpm) for 24 h at 25 °C. Initially, a concentration of 100 μmol L^{-1} for the solution of DPPH$^{\bullet}$ in only methanol was used. In order to analyze solvent effects, the concentrations of 100 μmol L^{-1} for MGN and 50 μmol L^{-1} for DPPH$^{\bullet}$ were used.

2.5. Radical scavenging activity of 2,2-diphenyl-2-picrylhydrazyl radical (RSA-DPPH$^{\bullet}$)

The antioxidant activities of MGN, β-CD, MGN:β-CD complex samples and GA (positive control) were measured in terms of their radical scavenging ability (RSA), using the DPPH$^{\bullet}$ method. MGN, β-CD or MGN:β-CD complex solutions (0.30 mL) were mixed with 2.7 mL of 50 μmol L^{-1} DPPH$^{\bullet}$ solution in different proportions of methanol:water and ethanol:water (20:80, 30:70, 50:50 and 100:0 mL:mL) in a 3 mL-quartz cuvette. The DPPH$^{\bullet}$ absorption values were obtained at 516 nm every 5 min, during 50 min by UV–vis spectrophotometer (MultiSpec-1501, Shimadzu, Japan). The results are expressed as remaining DPPH$^{\bullet}_{R}$ (%) as a function of time (Oliveira et al., 2009). All measurements were performed in triplicate.

2.6. Oxygen Radical Antioxidant Capacity-Fluorescein (ORAC-FL) assays

The MGN, β-CD and MGN:β-CD (1:1) complex aqueous solutions were prepared with 5 mL ethanol/100 mL water at a concentration of 100 μmol L^{-1}. The solution was stirred (170 rpm) for 24 h in the absence of light. The ORAC analyses were carried out on a Synergy HT multidetection microplate reader, from Bio-Tek Instruments, Inc. (Winooski, USA), using 96-well polystyrene white microplates, purchased from Nunc (Denmark). Fluorescence was read from the top, with an excitation wavelength of 485/20 nm and an emission filter of 528/20 nm. The plate reader was controlled by Gen 5 software. The ORAC was determined as described by Ou, Hampsch-Woodill, and Prior (2001), with slight modifications. The reaction was carried out in phosphate buffer (pH 7.4, 75 mmol L^{-1}): 150 μL of Fluorescein (FL, 40 nmol L^{-1}, final concentration) and 25 μL of free or complexed MGN solutions were placed into the microplate wells and pre-incubated for 15 min at 37 °C, thereafter 25 μL of the AAPH solution (18 mmol L^{-1}, final concentration) were added. The microplate was immediately placed in the reader and the fluorescence was recorded every 1 min for 90 min. A blank with FL and AAPH, using water and ethanol instead of the antioxidant solution, and five calibration solutions using Trolox (0.5, 1.0, 1.5, 2.0 and 2.5 μmol L^{-1}) were also used in each assay. The inhibition capacity was expressed as Trolox equivalents (mol L^{-1}) and was quantified by integration of the area under the fluorescence decay curve (AUC). The ORAC value was calculated by plotting the net AUC against the concentration as described by Folch-Cano, Jullian, Speisky, and Olea-Azar (2010).

2.7. Antioxidant capacity in membrane biomimetic system

Unilamellar vesicles of soy phosphatidylcholine (1 mmol L^{-1}) were prepared by extrusion (100 nm pore diameter membrane, at 25 °C) in 10 mL of phosphate buffer (50 mmol L^{-1}, pH 7.4 with the additional incorporation of 0.1 μmol L^{-1} of the peroxyl-sensitive

fluorescent probe C_{11}-BODIPY$^{581/591}$ as described by Oliveira et al. (2009)). The particle size was confirmed by Nanotrac-Zetatrac, NPA151-31A-0000-D30-10M model being around 100 nm.

Fluorescence measurements were carried out at 37 °C using a RF-5301PC spectrofluorophotometer (Shimadzu, Japan). In a 1 mL-quartz cuvette, adequate amounts of the unilamellar vesicle suspension, of the phosphate buffer pH 7.4, and of the sample (100 μmol L^{-1} MGN or MGN:β-CD complex) or Trolox (100 μmol L^{-1}), as a positive control, were mixed. The β-CD aqueous solution and buffer were used as negative controls. The reaction was initiated with the addition of 100 μL of AAPH (100 mmol L^{-1}). The fluorescence decay ($\lambda_{excitation}$ = 580 nm, $\lambda_{emission}$ = 600 nm) was continuously monitored over 30 min.

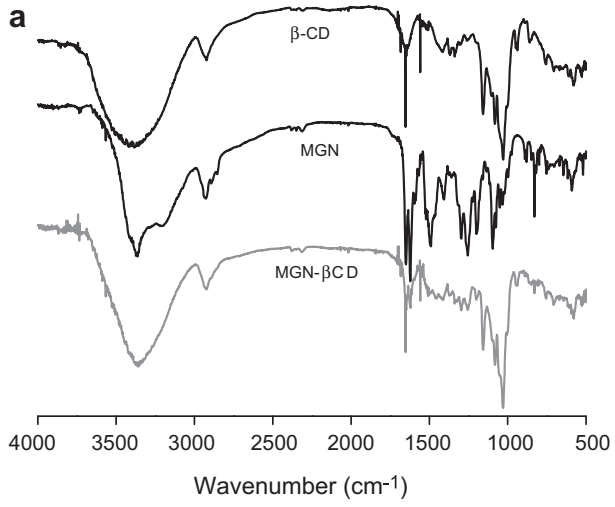
3. Results and discussion

3.1. Physicochemical characterization of MGN:β-CD complex

3.1.1. FT-IR

The FT-IR spectrum of β-CD (Fig. 2a) showed absorption bands at 3400 cm^{-1} (for O–H stretching), 2927 cm^{-1} (for C–H stretching) and 1157, 1082 and 1028 cm^{-1} (C–H, C–O stretching), as shown in the amplified spectra (Fig. 2b). For MGN (Fig. 2a), absorption bands of the hydroxyl group (3373 cm^{-1}) and C–H asymmetric stretching at 2933 cm^{-1} were observed, while in Fig. 2b, an aromatic conjugated carbonyl group can be observed at 1651 cm^{-1} together with signals of aromatic nucleus (1622, 1492 (C=C), 1407 cm^{-1}). Bands at 1255 and 1093 cm^{-1} are attributed to –C–O and C–O–C stretching, respectively (Fig. 2b) (Abu-Yousef, Gunasekar, Dghaim, Abdo, & Narasimhan, 2011).

The interaction between MGN and β-CD was confirmed by FT-IR spectroscopy. By comparison of the spectra of the complex with the one from β-CD and MGN (Fig. 2), the observed major changes occurred in the region from 1800 to 500 cm^{-1} (Fig. 2b). Some IR bands of MGN had disappeared completely (1492, 1407 and 1093 cm^{-1}) or had their intensities altered (1651, 1622, 1296, 1255, 1199 and 829 cm^{-1}). In the complex, bands at 1651, 1622 and 829 cm^{-1} were observed, confirming the presence of MGN. Ferreira et al. (2010) showed that the NMR signals for H-5 and H-8 (Fig. 1b) of MGN in the complexed form underwent downfield shifts from 6.8 to 6.9 δ and from 7.4 to 7.6 δ, respectively, indicating that the aromatic hydrogen signals are influenced by the presence of β-CD in the medium.



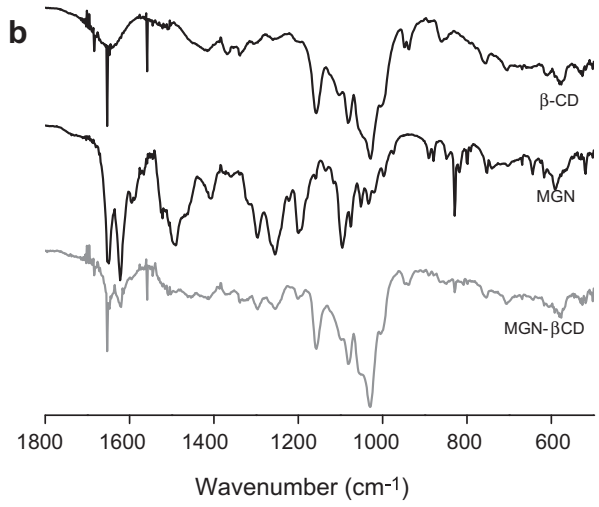


Fig. 2. a) FT-IR spectra of MGN, β-CD and MGN:β-CD complex obtained by co-evaporation method; b) amplification of FT-IR spectra at 1800–500 cm^{-1} region of MGN, β-CD and MGN:β-CD complex.

3.1.2. Differential scanning calorimetry (DSC)

The thermal curves of MGN, β-CD and the MGN:β-CD complex are shown in Fig. 3. The DSC curve of MGN (Fig. 3a) displayed one sharp fusion endothermic peak close to 252.6 °C, corresponding to the melting point. Neelakandan and Kyu (2009) found the melting temperature of MGN around 260 °C using the DSC technique. After MGN melting, the DSC curve indicated a thermal stability until 400 °C. In the case of β-CD (Fig. 3b), a broad endothermic signal was observed around 88.8 °C, correspondent to water loss by evaporation ($t < 100$ °C). A sharp endothermic signal was observed close to 295 °C corresponding to the melting point of β-CD, followed by endo-exo effects that are related to thermal degradation in 335 °C. These results agree with literature data (Giordano, Novak, & Moyano, 2001).

DSC curve of the physical 1:1 mixture of MGN and β-CD (Fig. 3c) was a superimposition of individual components of MGN and β-CD. An endothermic signal correspondent to the fusion of MGN suffered displacement from 253.0 °C to 255.2 °C, and the fusion temperature of β-CD experimented a reduction from 335.0 °C to 271.4 °C.

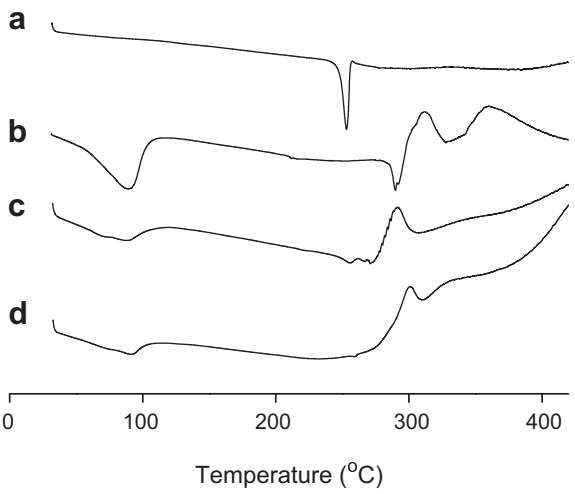


Fig. 3. DSC thermograms of: a) MGN; b) β-CD; c) MGN and β-CD physical mixture in a molar ration 1:1 and d) MGN:β-CD (1:1) complex obtained by co-evaporation method.

DSC curve of MGN:β-CD 1:1 complex (Fig. 3d) showed a broad endothermic peak between 80 °C and 100 °C corresponding to evaporation of water molecules absorbed on the lattice and/or inserted into β-CD cavities. DSC curves corresponding to pure β-CD, physical mixture and MGN:β-CD complex had shown that the amount of water was minor after the MGN incorporation. For the complex MGN:β-CD was observed that fusion endothermic peak of the MGN almost disappeared, however, a small endothermic peak was detected at 259.5 °C, which displacements confirmed that MGN was included into β-CD cavity.

3.2. Radical scavenging activity toward DPPH•

Fig. 4 shows the percentage of DPPH• radical-scavenging activity (RSA-DPPH•) of the samples, in comparison with the GA control. Note that the MGN:β-CD 1:1 complex showed a higher antioxidant activity when compared with its free form, for MGN concentrations of 50 and 100 μmol L^{-1}. As expected, GA was more effective. The highest MGN concentration of 500 μmol L^{-1} shows a RSA-DPPH• (%) similar to the one obtained with GA (100 μmol L^{-1}).

MGN in complexed form is more reactive toward DPPH• than its free form, except at higher concentration 500 μmol L^{-1}. At this concentration, MGN is in excess in the medium consuming all DPPH. For the MGN:β-CD (1:1) complex at a concentration of 50 μmol L^{-1}, the value of RSA-DPPH• (%) is about 65%, while for its free form, it is about 28%. In the case of the complex at 100 μmol L^{-1}, the value increased to 78%. The same assay was performed with β-CD alone and no significant antioxidant activity toward DPPH• was observed, as described by Lu et al. (2009).

It is noticeable that the effect of β-CD on RSA-DPPH• was more pronounced at a low concentration of MGN. Several authors studied the complexation of cyclodextrins with polyphenols with evidence of increase in their antioxidant capacity (Alvarez-Parrilla, de La Rosa, Torres-Rivas, Rodrigo-Gracia, & González-Aguiar, 2005).

In the present work, it was observed that the antioxidant activity of MGN is influenced by β-CD. The antioxidant activity of MGN is increased after complexation with β-CD (at 50 and 100 μmol L^{-1}). According to Dar et al. (2005), positions 6 and 7 (Fig. 1b) of MGN are mainly responsible for its antioxidant property. Ferreira et al. (2010) showed that the NMR signals for H-5 and H-8 (Fig. 1b) of MGN in the complexed form underwent downfield shifts from 6.8 to 6.9 δ and from 7.4 to 7.6 δ, respectively, indicating that its aromatic hydrogen signals are influenced by the presence of β-CD in the medium, increasing the antioxidant activity of the MGN:β-CD complex. A possible rational for this enhancement is based on the following equilibrium reaction:

$$\mathrm{MGN} + \beta - \mathrm{CD} \rightleftharpoons \mathrm{MGN} : \beta - \mathrm{CD}$$

The reaction between MGN and DPPH can occur in solution, i.e. mangiferin goes out of the cavity (maintaining a close proximity), undergoes the process of oxidation and then its oxidized form search stability in the cavity of β-CD. It is also worth remembering that even though the 6-OH and 7-OH are the most important groups concerning the antioxidant activity of MGN, 1-OH and 3-OH (out of the cavity) are also likely to suffer the oxidation process (Gómez-Zaleta et al., 2006).

Initially, for the DPPH• assays, methanol was used as a solvent. Some authors (Lucas-Abellán, Mercader-Ros, Zafrilla, Gabaldón, & Núñez-Delicado, 2011) criticize the use of large amounts of organic solvents when using this DPPH• assay to evaluate antioxidant activity of substances complexed with cyclodextrin. Thus, a study of the solvent effects on the antioxidant activity of MGN was performed, using methanol–water and ethanol–water. The concentrations used were 100 μmol L^{-1} for MGN and 50 μmol L^{-1} for DPPH•.

Fig. 5 shows the solvent effects on the antioxidant activity of MGN, for its complex 1:1 and for GA. It is not possible to use a percentage of solvent lower than 50%, because DPPH• precipitates in the medium, due to its insolubility in water, as already described by Li, Zhang, Chao, and Shuang (2009). Some authors use only organic solvent to determine the antioxidant activity of complexes with CDs, as cited by Strazisar, Andrensek, and Smidovnik (2008) and Lu et al. (2009).

Lucas-Abbellán et al. (2011), affirm that DPPH• cannot be used to measure the antioxidant activity of complexes with CDs, because the methanolic medium of the DPPH• assay prevents complexation in the hydrophobic cavity of CDs. However, in our work, it was observed that the antioxidant activity of MGN:β-CD increases as the amount of methanol and ethanol increases, reaching a maximum when only organic solvent is used, with this increase being more pronounced for ethanol. For confirmation purpose, another method was used for quantifying the antioxidant activity for instance, ORAC (Folch-Cano et al., 2010).

3.3. ORAC-fluorescein (ORAC-FL) assay

The ORAC-FL assay consists of measuring the decrease in the fluorescence of FL when it suffers peroxyl radical-based oxidative damage (Lucas-Abellán et al., 2011).

Once the antioxidant activity of MGN had been established, our purpose was to demonstrate the effect of its inclusion on β-CD on the same activity, by using the ORAC-FL assay. Fig. 6 displays the results in terms of the respective areas under the curve: The kinetic profiles obtained for free MGN (Fig. 6a) were smaller than those obtained for MGN:β-CD complex (Fig. 6b). The ORAC values were obtained by plotting net AUC *vs.* concentration of free or complexed MGN (Fig. 6a and b, insert). The ORAC value was calculated as indicated in Section 2.6, and the results showed that MGN alone has an ORAC value of 2.3, it means 2.3 times better than the standard molecule, trolox, while the MGN:β-CD complex shows an ORAC value fifteenfold larger.

The presence of induction time in ORAC-FL profile by addition of MGN was shown in Fig. 6a. This is related to the time in which the probe molecule, in this case, Fluorescein, is protected against oxidation produced by peroxyl radicals, in the presence of increasing amounts of antioxidant molecule. For the MGN:β-CD

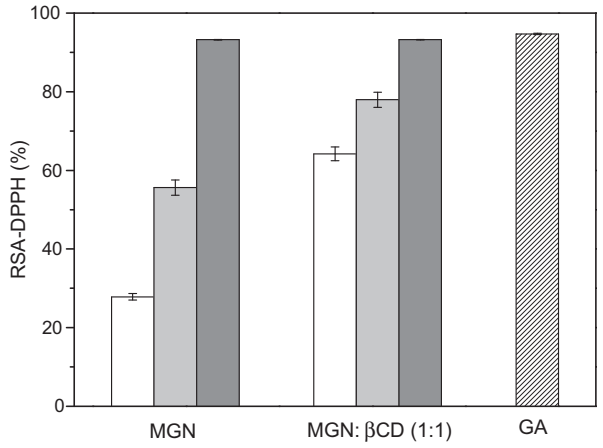


Fig. 4. Percentage of RSA-DPPH• of MGN and MGN:β-CD 1:1 complex and gallic acid (GA). Time of reaction with DPPH•: 30 min. For MGN columns, white: 50 μmol L^{-1}; gray: 100 μmol L^{-1} and dark gray: 500 μmol L^{-1}. Gallic acid: 100 μmol L^{-1}. DPPH: 100 μmol L^{-1} in only methanol.

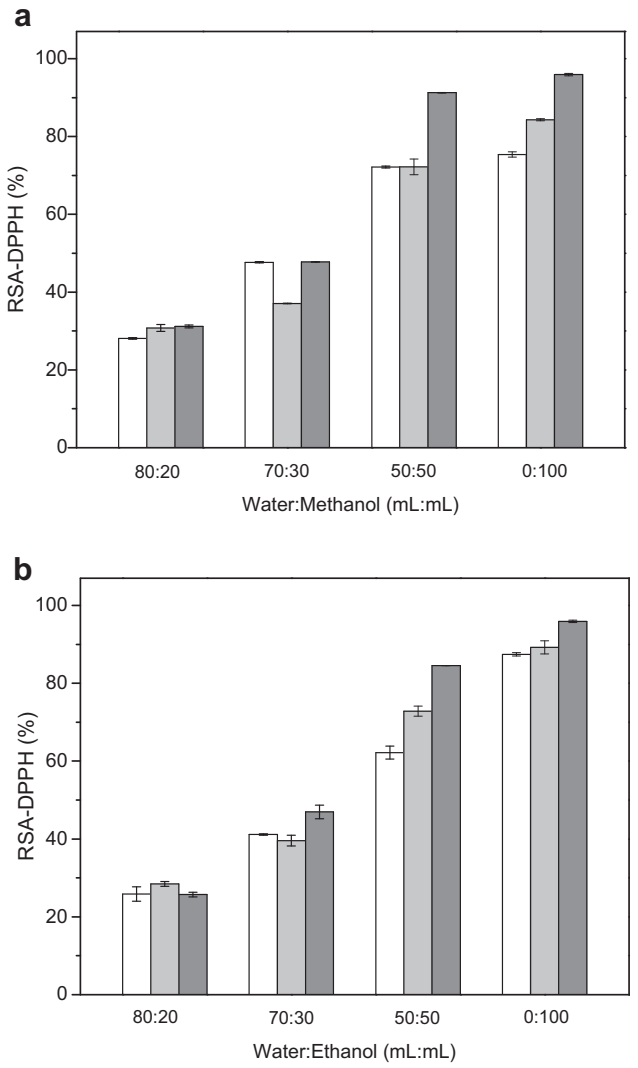


Fig. 5. Percentage of RSA-DPPH˙ of the samples as function of the type of solvent ((a) methanol and (b) ethanol) and amount of solvent. Time of reaction with DPPH˙: 30 min. For columns, white: MGN 100 μmol L^{-1}; gray: MGN:β-CD complex (1:1) and dark gray: gallic acid 100 μmol L^{-1}. DPPH: 50 μmol L^{-1}.

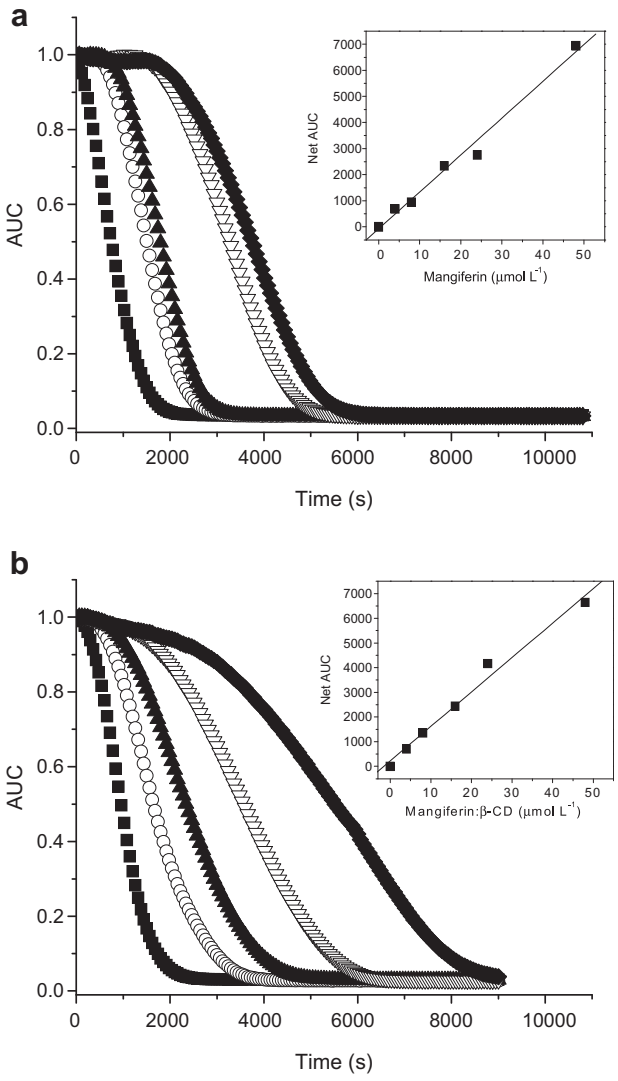


Fig. 6. a) ORAC-FL curves in the presence of MGN in a concentration range 0–50 μmol L^{-1}. Insert: AUC_{NET} *vs.* MGN concentration graph; b) ORAC-FL curves in the presence of the MGN:β-CD complex in a concentration range 0–50 μmol L^{-1}. Insert: AUC_{NET} *vs.* MGN:β-CD complex concentration graph.

complex, a higher protection was observed. The combined results show that the antioxidant activity of MGN is influenced by the presence of β-CD. Lucas-Abellán et al. (2011) and Folch-Cano et al. (2010) found similar results using the ORAC assay, when phenolic compounds were complexed in CDs. According to Lucas-Abellán et al. (2011) ORAC is the best method for quantifying the antioxidant activity of phenolic compounds, when complexed in CDs, because FL and AAPH do not suffer interference with CDs complexation. The ORAC method was adequate to measure the antioxidant activity of MGN:β-CD complex, but does not show the capacity to inhibit oxidation and lipid peroxidation (Niki, 2010). Thus, we evaluated the behavior of MGN and the MGN:β-CD complex by the method of lipid peroxidation.

3.4. Lipid peroxidation measurements

The protective effect of antioxidants against lipid peroxidation has been studied extensively (Niki, 2010). It has been shown that the capacity of free radical scavenging by antioxidants does not necessarily correlate with the capacity of inhibition of lipid peroxidation. Therefore, it is essential to evaluate the protective effect of antioxidants against lipid peroxidation.

The lipid peroxidation protection was evaluated using a peroxyl radical-mediated lipid peroxidation membrane model (soy lecithin unilamellar liposomes) as described in Section 2.7. Fig. 7 displays a graph from lipid peroxidation protection (%) *versus* time (total time 30) of the systems: phosphate buffer (negative control), β-CD (negative control), Trolox (positive control), MGN:β-CD (1:1) complex, and MGN. Liposome with C_{11}-BODIPY$^{581/591}$ and AAPH (generator of peroxyl radical) were added in all cases.

AAPH-induced liposomal lipid peroxidation was observed in the absence of antioxidants (negative controls, Fig. 7). The antioxidant ability of trolox is well-known and it inhibits lipid peroxidation. Lipid peroxidation assays have shown that MGN and MGN:β-CD complex have protective effects on the membrane as well as trolox, which is different from other results in the literature that have shown that the complexation between the substrate and CD can decrease antioxidant ability, as observed for carotenoids (Polyakov, Leshina, Konovalova, Hand, & Kispert, 2004). The protective effect herein found is relevant, since the inclusion of MGN in β-CD favored the membrane protection.

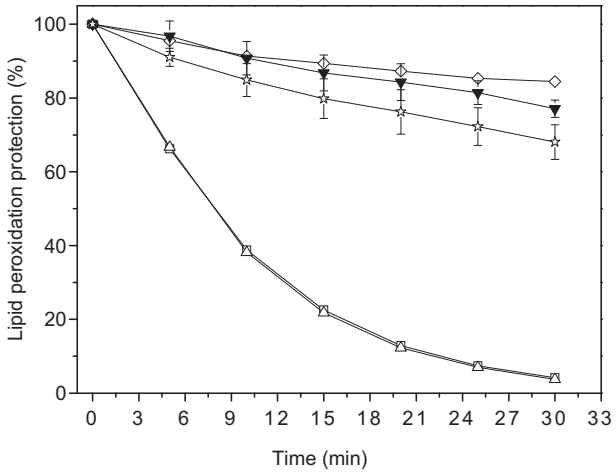


Fig. 7. Lipid peroxidation protection (%) afforded by -✶- MGN (100 μmol L^{-1}) and -▼- MGN:β-CD (100 μmol L^{-1}) complex and a positive control (-◇-Trolox, 100 μmol L^{-1}). -□- Phosphate buffer and -△- β-CD were used as negative controls.

4. Conclusions

The results of FT-IR and DSC demonstrated that the MGN:β-CD complex has different physicochemical characteristics when compared with free MGN. Thus, MGN was efficiently complexed in the β-CD cavity by the co-evaporation method. Present results have shown that the antioxidant activity of MGN is increased in the presence of β-CD. This was confirmed using ORAC-FL and DPPH• assays. The ORAC-FL method was more effective in the evaluation of the antioxidant activity of MGN in a CD complex. The study of the solvent effects for the DPPH• method demonstrated that at amounts of organic solvents lower than the ratio 50:50 (methanol–water or ethanol–water) it is impossible to use this method, due to the hydrophobic nature of DPPH•. It is essential to use several methods to evaluate the antioxidant activity and protective effect of a lipophilic antioxidant complexed in CDs. Lipid peroxidation assays have shown that MGN:β-CD complex have protective effects on the membrane as effective as the positive control (trolox). In the future, MGN, encapsulated with β-CD, can be used in order to control its release *in situ* for enhancement of therapeutic effects. Industrial applications of MGN and its complex in the food industry are also expected.

Conflict of interest

There is no conflict of interest.

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